understand how these changes shift the balance towards cartilage destruction and to undertand the interactions between the cartilage and the sunchondral bone.
Methods: Extraction of good quality RNA from joint tissues has been one of the major problems for microarray analysis. We have developed a RNA extraction method for extracting RNA from tissues stored in liquid nitrogen from cartilage as well as the subchondral bone. The method for extracting RNA from specific areas from different regions of the tibia plateau was also developed. This new method has allowed us to identify regions of different severity for OA on the same individual, thereby reducing the background causing by analyzing microarray data from different individuals. Using morphological analysis and OARSI scoring, the knee tibia plateau was divided into four regions representing four different stages of OA: the meniscus covered region in the external lateral tibia as early OA (average OARSI scoring = 5.7); the anterior lateral tibia as intermediate OA (average OARSI scoring = 11.75); the medial tibia with remaining cartilage as late stage OA (OARSI scoring = 15) and regions with complete loss of cartilage as final stage of OA. RNA extracted from cartilage and subchondral bone from these regions were evaluated by whole genome oligonucleotide microarray analysis (n=10). RNA from corresponding regions in non-OA normal control (n=2) were also included.
Results: Preliminary data showed that the RNA extracted using the developed can be used for microarray analysis. Using the early OA as a references, the other three regions revealed approximately about 100 genes showed ≥ 2-fold differences in expression between the cartilage tissue pairs. Most of these genes related to the extracellular matrix synthesis, cell proliferation and interstitial collagen synthesis, suggesting genes might play key roles in breakdown of cartilage homeostasis. One interesting observation was that many of these genes show temporal expression suggesting there is a complex interaction and regulation.
Conclusions: Previous microarray studies only allows comparison between early and late stages of OA, how the genes changed in between could not be observed. The method developed from this study has allowed us to look at this largely ignored gap. Using the normal samples as a reference, it is anticipated that the earliest changes could be detected using this method.

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EVALUATION OF AGE OF SYMPTOMS ONSET ON THE ASSOCIATION OF SUSCEPTIBILITY LOCI WITH KNEE OSTEOARTHRITIS

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Purpose: It is common to find in genetic studies of complex diseases that patient with a younger age of disease onset show a more marked effect of genetic susceptibility factors. We do not know if that is also the situation in osteoarthritis. However, it seems congruent with what we know about its mechanisms. It seems more likely that susceptibility alleles will favour earlier development of the disease, whereas a longer exposure to environmental factors or joint overload linked to profession, repeated trauma or overweight, will be required in subjects with less genetic predisposition. Therefore, we aimed to test the effect of age of onset of clinical symptoms in the frequency of susceptibility alleles for knee osteoarthritis.
Methods: We selected five polymorphisms that have been associated with knee osteoarthritis in the most convincing way: rs143383 (GDF5), rs4140564 (PTGS2), rs3815148 (GPR22), rs12535761 (GPR22), rs7639618 (DVWA), and a microsatellite in ASPN. The SNPs were genotyped by minisequencing. The microsatellite alleles were assessed in a capillary sequencer by the size of the amplification products using a FAM-labelled primer. Genotypes were obtained in 262 patients that have undergone total knee replacement (TKR) because of primary osteoarthritis. Age of symptoms onset was the reported by the patients. Results were stratified into carriers of the risk allele and non-carriers. The age distributions of these strata were compared graphically with Q-Q plots and by the Student's test. We also performed combined analysis using linear regression between age of symptoms onset and the number of risk alleles in the five polymorphisms carried by each patient
Results: There was not any trend in the direction of a younger age of symptoms onset in the patients carrying the risk alleles. This lack of trend was observed in the graphic analyses, in the Student's T tests for each polymorphism, and in the combined analysis of all the risk alleles.
Conclusions: We did not find any significant correlation between carrying risk alleles for knee osteoarthritis and age of symptoms onset. This result is surprising and requires a review of our concepts about the interplay of age, environmental and genetic factors on the onset of clinical osteoarthritis.

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IMPORTANCE OF THE SELECTION OF CONTROLS IN GENETIC STUDIES OF KNEE OSTEOARTHRITIS

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Purpose: Genome Wide Association studies have provided data on hundreds of thousands polymorphisms for large collections of population controls. They can be used to compare with genotypes of patients with low prevalence diseases and there is abundant experience of the validity of this approach. However, it is unclear to what extend the use of these data will be efficient for the study of a prevalent disease as osteoarthritis. If they provide a too low power, the study of specifically selected controls will still be required. Therefore, we aimed to determine the effect of using controls selected with different levels of stringency on the association of known knee OA susceptibility polymorphisms.
Methods: We selected five polymorphisms that have been associated with knee osteoarthritis in the most convincing way: rs143383 (GDF5), rs4140564 (PTGS2), rs3815148 (GPR22), rs12535761 (GPR22), rs7639618 (DVWA), and a microsatellite in ASPN. The SNPs were genotyped by minisequencing. The microsatellite alleles were assessed in a capillary sequencer by the size of the amplification products using a FAM-labelled primer. Genotypes were obtained in 262 patients that have undergone total knee replacement (TKR) because of primary osteoarthritis. They were compared with two sets of controls. The largest included 1393 subjects older than 55 years and without any surgery related with joint diseases (CRL). A subset made of 470 of these patients were in addition free of any lower limb OA symptoms according to their responses to a questionnaire (B-CRL = best controls). Assessment of the effects of the selection criteria on the power of comparisons between TKR and the two groups of controls was done with the program "Power and sample size" developed by Dupont and Plummer.
Results: The increase in OA risk contributed by each of these polymorphism was small, but all of the risk alleles were more common in TKR patients than in the B-CRL group. Mean Odds Ratio of allele frequencies of the 6 polymorphisms were 1.09 when comparing TKR patients with B-CRL and 1.05 when comparing TKR patients with the whole CRL group. This difference in effect size is small, but it had dramatic consequences in statistical power (Figure 1: B-CRL = blue line; CRL = red line). This differences were observed with the parameters of the study, showing that the lower power of comparisons with CRL could not be compensated by the larger sample size of the CRL group (about 3× larger than the B-CRL subgroup). They will not be compensated even by a much larger excess of controls over the number of patient (red dashed line = 100× excess of CRL).

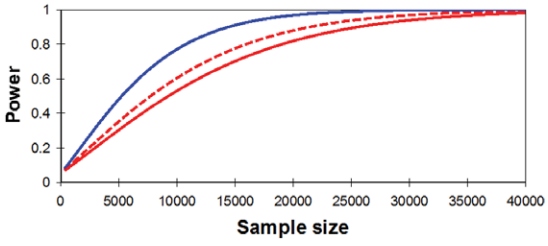

Figure 1

Conclusions: Our results suggest that using population controls with poor selection or without selection in genetic studies of knee osteoarthritis will be very inefficient. This is due to the small effect sizes of the risk alleles, which make associations very intolerant to even the lowest additional reduction in effect. However, the very same nature of these small effects demands further studies for confirmation.